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Cell union in herbaceous grafting.

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WITH PLATES XXX AND XXXI.

The plants mainly employed in this study were tomatoes, potatoes and geraniums, although some work was done with cactus, tradescantia, and a few other kinds of plants. The entire work was done in the winter and early spring of 1892, the experiments being performed in the greenhouse attached to the laboratory of vegetable physiology of Purdue University.

Three methods of grafting were employed, inarching, splice grafting and cleft or wedge grafting. It is needless to describe these well known methods here farther than to say that by the inarching method the scion is allowed to remain on the parent stock until union is formed, while by the splice and wedge or cleft methods the scion must from the start sever all connection with the parent and be sustained entirely by the new stock. In plants which are very delicate and of slow or uncertain union, the inarching method is the surer, though in most cases the cleft and wedge are safe and most convenient.

In all cases the stock and scion were held in place by thin strips of raffia until union was accomplished when they were removed to allow the diameter of the stem to increase.

It is only in certain stages of growth that herbaceous plants may be easily grafted; in quite young plants the tissues are not strong enough to survive the injuries inflicted in the operation, while in older parts, those past or nearly past the growing stage, the union does not take place readily because of the scarcity of meristematic tissue, as union depends upon the active growth of this tissue. In herbaceous grafting the rule is to use only such stems as will snap in breaking and not crush at the point of yielding. The scions used in this work were in all cases vigorous growing young tips. In young plants the stocks were cut close to the ground, in older plants higher branches served the purpose.

In the experiments it was noticed that scions which had grown in vertical position more readily united with stocks which allowed them to retain their original position. Shortly after the operation of grafting many scions wilt and remain in a drooping state several hours or even a day, but with careful attention revive permanently.

Accounts were kept of each graft and when it arrived at desired age for study the parts at the point of union were cut into small pieces suitable for sectioning, dehydrated in a Thomas apparatus and prepared for sectioning by the celloidin method.

Although all grafts were recorded so that sections might be had showing grafts at various ages and all stages of cell union, study of sections showed that in most instances all stages of cell union could be found in a single graft. Longitudinal and transverse sections were made of most grafts, but the longitudinal section seldom showed anything in addition to that shown by transverse sections. Camera lucida drawings were made of all sections of importance, and in connection with the slides used for study. A number of these drawings have been selected to illustrate this article.

Graft of tomato on tomato.—Two lateral and parallel tomato branches belonging to separate plants had tangential slices, each about three-fifths of an inch long, removed from their adjacent sides; these cut surfaces were bound firmly together with raffia so that similar tissues met. After about four weeks the graft was sectioned for study. The cross section showed the line of union marked by a ragged brownish wall which passed with but one interruption entirely across the stem. In longitudinal section this junction of the two members was marked by rows of small irregular parenchyma cells with here and there the intervention of a thickened brown wall (fig. 1). The sections showed that the cells which had been injured in grafting had died, while those immediately beneath them were stimulated to a vigorous growth forming meristematic tissue in each member, which in its advance pushed the broken walls of the dead boundary cells into line, forming between the two members of the graft the fragmentary brown wall noted in the section (fig. 2, *l,l*). This brown wall in all the instances observed was unaffected by stains used on the section. As will be seen farther on, this wall tends to disappear with age until at last only a mere trace of its existence is left.

In this case the tangential slices, removed in bringing the stock and scion into shape for grafting, carried with them a part of the woody zone of each internode, so that in cross-section the remaining part appears horseshoe shaped. In binding the scion to stock the tips of these woody rings were bound closely together (fig. 3), but in process of union parenchymatous tissue developed and intervened between the woody zones of the two members, resulting in their wide separation, as seen in figs. 2 and 4.

In sections of potato grafted to potato no new points were noted, the union occurring by the process described in the previous case, except that the brown wall which so strongly marks the junction in the case of the tomato was not nearly so prominent.

Potato and tomato graft.—The cross section of a wedge graft of a potato to a tomato, five weeks old, showed that similar tissues in stock and scion had met in the union. At the time of grafting numerous gaps and small spaces existed between the two members of the graft owing to the irregularities and unevenness of the cut. The parenchyma cells of stock and scion had elongated towards these places, being the direction of least pressure, and had filled up most such cavities, the line of meeting of stock and scion tissues being indicated by a wall twice the thickness of the ordinary cell wall. In other places this wall was very ragged and fragmentary due to dead cell walls which had been crowded into line by the growing tissues; shown in fig. 5.

The growth of the cells in closing up gaps is similar to growth of meristematic tissue induced by surface wounds. The cells just outside the path of the knife elongated in the direction of the severed surface to double the ordinary dimensions or more, then formed a series of transverse walls making what appeared in cross section to be series of narrow plate-like cells, the larger walls of which were parallel to the cut surface, the longer dimension being equal to the width of the cell from which they originated. (See figs. 5, 11, etc.) At the outer part of the section the central parenchyma of the stock was shown to have met the cambium of the scion while the cambium of the stock met the parenchyma of the primary cortex in the scion. The union here was easily accomplished through development of meristematic tissue. The section showed the central parenchyma to have been more active in forming a union than was the primary cortex.

In one case where a potato scion had been grafted to tomato stock the central parenchyma of the tomato stock was bound against soft bast cells of the fibrovascular bundle. The bast cells had swollen as if attempting to enlarge, the cambium of the vicinity began an active growth, and a considerable quantity of meristematic tissue intervened between the central parenchyma and the bast cells of the fibrovascular bundle (fig. 6).

In one instance the knife in preparing the parts for grafting had removed one side of a cell in the potato scion, while a cell of the stock had suffered a similar loss, and it happened that the free portions of the severed walls of these two cells were bound end to end. Through continued pressure the two partial cells had united, forming a cell twice the normal size, which lived and grew. This large cell, however, threw a delicate wall across its middle, forming two cells each of normal size, and providing such a neat union between stock and scion that it was almost impossible to detect it, the path of the knife showing no thickened wall, with but here and there a few fragments of dead walls projecting into the newly formed cell. See fig. 7.

The graft which presented the above interesting union also showed in an admirable way the action of parenchyma cells in closing up spaces existing, immediately after the operation, between members of the graft.

As previously stated the removal of pressure from one side of the cells both stimulates growth and induces elongation of the cells in the direction of least resistance. In this graft the cells had elongated towards the injured surface until their length was four times their width; delicate walls were then passed transversely across the cells dividing them into a series of small cells. These transverse walls appeared near the free end of the cell at first, and when any variation of size occurred in the smaller cells, as was often the case, the one most remote from the injured surface was the largest. Close examination revealed, in many instances, delicate young walls pasing through the young cells at right angles to their longer axes. The free sides of these growing cells were much more thickened than the others (fig. 8).

The cross section of another graft of potato on tomato showed the central parenchyma of the tomato joined to the soft bast, cambium and fibrovascular bundles of the potato. Although the line of union was marked by a slightly thickened wall, no gaps were present and no development of meristematic tissue occurred. The junction of the two members was so neat as to be no more marked than the transition from one tissue to another in the same plant (fig. 9).

Geranium grafts.—Geraniums were grafted to each other with great ease. The action of these cells in effecting union, with occasional modifications, was similar to that seen in previous grafts of potatoes and tomatoes. Thickened brown walls generally marked the junction of the tissues, as in the former grafts; in some instances where gaps existed in this wall the cells of the stock and scion had so grown together as to wholly obliterate the path of the knife. In such places the longer axes of the cells were nearly at right angles to the line of junction (fig. 10).

Geranium and tomato grafts.—Geranium scions were easily united to tomato stocks, the geranium in nearly every instance thriving and increasing in foliage. The tomato scions would in no instance, however, accomplish union with a geranium stock. The repeated trials proved that in respect to grafting the two plants do not act reciprocally. As a partial explanation of this two facts are offered, viz: difference in the relative quantities of sap in tomato and geranium, and difference in acidity. The geranium has relatively much less sap than the tomato and in addition to this the sap is of greater acidity, so that in grafting osmotic action is set up between the tomato scion and geranium stock in which the tomato loses sap and at a time when it is least able to stand it and consequently perishes; in case of geranium scion and tomato stock the osmotic action would result beneficially to the scion supplying it with more sap at a time when it was in greatest need. Cross sections of these grafts showed the geranium to have been more active in the formation of the union (fig. 11).

Geranium and potato grafts.—Successful grafts were made with geranium scions to potato stocks. In this case as in the previous one the geranium was more active in forming the union, as shown by the cells along the line of junction of the two members (fig. 11). In this graft the wall which marked the line of union was thickened but otherwise similar to ordinary cell walls.

Cactus grafts.— Cactus grafts were made, and sections showed the method of union to be essentially like that in the other cases studied. The union occurred in either one or the other of two ways: long continued pressure holding cell walls in contact gradually causing them to cohere, or through the development of meristematic tissue by each member.

Grafts of a monocotyledonous plant.—The only monocotyledonous plant experimented upon was *Tradescantia zebrina*, which was grafted to itself with great ease; and with much surprise it was also found to form a true union with a tomato stock. In grafting tradescantia to itself the members of the graft were firmly bound, and healing was rapidly accomplished. The examination of cross sections showed no general development of meristematic tissue so prominent in previously described grafts. Those parts of boundary cell walls which were on the line of union were very much thickened and from all appearances most of the union had been effected through long exerted pressure which caused the cell walls permanently to cohere. Where gaps or spaces had existed however elongation of cells in that vicinity had occurred as in other grafts. Where parenchyma met a fibrovascular bundle, and a space existed between the two, the parenchyma cells elongated towards the gap and divided, giving rise to new cells until the space was closed and the union accomplished through pressure of the cell walls as in previous cases.

Union of tradescantia and tomato.—In cross section of a graft of tradescantia upon tomato the union was found to be as perfect as any between tomato stock and potato scion. In many places tissues of both members gave evidence of marked activity in forming union, in others the outlying walls of border cells were thickened and union here was shown to have been the result of pressure of the border cell walls upon each other. The tomato was the more active in forming the union (fig. 12).

Examinations of callus.—Examinations of callus in tomato and potato slips, which had been placed in damp sand, were made in order to see what relation exists between the healing of these external wounds, and of the internal wounds of grafts.

The short account here given is from the examination of potato callus, as it showed all the parts clearly. From a longitudinal section it was seen that all the cells injured by the knife died, the parenchymatous tissues immediately beneath were vigorous and were stimulated to renewed growth. Elongation towards the cut surface occurred in the cells, and by forming successive transverse walls each elongated cell gave rise to several small cells, which rapidly increased in thickness of cell wall, and became rounded. These small cells pushed the dead cells in advance as they grew, and soon enveloped the entire injured tip and extended upward enveloping the sides of the branch with a mass several layers thick. The walls of the callus cells are thicker than those of the parenchyma cells, which give rise to them, and the cell contents are richer in protoplasm. The outer callus cells give rise to root hairs (fig. 13).

At the tip the transition from callus cells through meristematic tissue into ordinary central parenchyma is very gradual. The examination of the tip showed the first stages in the formation of callus to be similar to those in effecting union of tissues by grafting. Hansen, who has carefully studied the formation of callus, regards a cut made in a stem, when vegetative conditions are favorable, as a stimulus to extensive and complex activities. In the cut necessary to grafting we have the stimulus to growth, the parenchyma cells respond to the stimulus by developing meristematic tissue which in most cases effects the union.

More or less true callus may be found in the graft serving to protect injured parts which by reason of their position could never unite. If proper conditions of moisture be present the graft also frequently gives rise to rootlets as does the callus. The union of tissues in the graft and formation of callus is similar. Like causes produce each. One is the healing of an internal the other of an external wound. The callus may exist on the graft, and parenchyma which in one place produces a callosity to protect an exposed injury, in an adjacent locality effects a union between stock and scion, the process in each case being similar.

Summary.—We find that union in herbaceous grafting occurs in one of two ways: It is accomplished either by long exerted pressure holding old cell walls together and gradually causing them permanently to cohere, or through the development of meristematic tissue by one or both members of the graft, after which the boundary walls meet and unite through pressure, somewhat as in the first case. We generally have part of the union in any particular case formed by the coherence of the walls of old cells, and the remainder formed by the growth of new cells arising, as in the case of callus, back of the cells injured by the knife.

Broken walls of the injured cells are thus pushed into line forming a brownish fragmentary wall marking the junction of the two parts of the graft. This wall tends to disappear with age; the smaller young cells along the junction of the two members enlarge rendering the line of demarkation between the two tissues faint.

In cases where the severed ends of two woody zones met, but were not sufficiently close to allow of union through coherence of cell walls, the two opposite ends of woody zones were enveloped by meristematic tissue arising from the cambium layer on the one side and the central parenchyma on the other. These two layers of meristematic tissue, meeting each other between the ends of the woody zones united as in the preceding cases, except no fragmentary wall separated them. When such a process occurred we had the woody zones of the two members of the graft separated by a layer of parenchymatous tissue similar in general form to a medullary ray. In cases where the woody cells met parenchyma cells for union they were often first enveloped by a growth of the neighboring parenchymatous tissue of the member of the graft to which they belonged.

It may be interesting to account for the transference of water from the stalk to the scion. The work of transferring water from one part of a plant to another is done largely by woody tissue, parenchyma being unable to do it. If parenchyma intervene between the woody tissues of stalk and scion throughout the graft the water supply of the scion is cut off. But this intervention did not occur throughout the graft where successful union was accomplished; while some sections did not show direct contact of the woody systems, others of the same graft always disclosed it. In dicotyledonous plants the binding of the graft nearly always brought the ends of the rigid woody rings in direct contact. In grafting of monocotyledonous to dicotyledonous plants, as in tradescantia and tomato graft the scattered woody bundles of the tradescantia were often seen in direct contact with the woody cells of the tomato, thus giving opportunity for the transferrence of water from the one to the other.

Naturally one would not expect union between plants of different orders, and the successful grafting of *Tradescantia zebrina* upon tomato and of geranium upon tomato was a surprise. The union of tomato, a dicotyledonous plant, and tradescantia, a monocotyledonous plant, was particularly interesting and points to the fact that a similarity in the arrangement of the woody and other tissues is not essential in grafting herbaceous plants.

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Explanation of Plates XXX and XXXI.

Abbreviations used: *l, l,* line of union; *G,* geranium; *T,* tomato; *P,* potato; *Td,* tradescantia.

Plate XXX.—Fig 1, Long. sec. of *T* to *T; n* young cells formed in process of union.—Fig. 2. Trans. sec. of *T* to *T; w, w,* woody zone; *cp, cp,* central parenchyma: *cp'* cortical parenchyma; *m,* young cells.—Fig. 3. Trans. sec. *T* to *T; w, w,* woody zones; position of *w, w,* shown at time of grafting.—Fig. 4. Trans. sec. *T* to *T,* showing position of *w, w,* after graft had healed.—Fig. 5. Trans. sec. *P* to *T,* through central parenchyma; *n, c,* young cells.—Fig. 6. Trans. sec. *P* to *T; cm,* cambium tissue; *b,* bast cells.—Fig. 7. Trans. sec. *P* to *T,* through central parenchyma; *n,* young cell walls; *a,* fragments of broken walls formerly occupying places of *n.*—Fig. 8. Trans. sec. *P* to *T,* showing growth of tissue and formation of young cells, *n, n,* in closing up gaps, *gp, gp; t, v,* young transverse walls dividing young cells; *t, t,* thickened walls of boundary cells.

Plate XXXI.—Fig. 9. Trans. sec. *P* to *T; sb,* soft bast cells; *c m,* cambium layer.—Fig. 10. Trans. sec. *G* to *G. m m,* newly formed cells; *n n,* distorted parenchyma cells.—Fig. 11. Trans. sec. *G* to *P; n,* young cells.—Fig. 12. Trans. sec. *Td* to *T; f, b,* fibrovascular bundle; *n,* young cells.—Fig. 13 Long. sec. callus formation. *c, c, c,* on potato slip: *w,* fibrovascular bundle; *b,* bast cells; *c, p,* central parenchyma: *br,* the broken walls of cells injured in cutting the slip; *k,* formation of callus cells by parenchyma cells; *h,* trichome arising from callus cells.